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The gelation of sodium alginate with calcium ions studied by magnetic resonance imaging (MRI)

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Abstract

The displacement of the sol/gel interface during the gelation of sodium alginate by calcium ions can be tracked in both one and two dimensions using magnetic resonance imaging. In the one-dimensional gelation experiments, the distance moved by the sol/gel interface was proportional to (time$^{1/2}$), which implies that the gelation process is diffusion-limited. Using the gelation model previously proposed by Smidsrød and co-workers, together with the initial reaction conditions and assuming that the diffusion coefficient of the alginate molecules is extremely small, it was found that the diffusion of calcium ions through the gel network is dependent on the initial concentration of the calcium, the ionic strength of the alginate solution, and the size of pores in the gel which is formed.

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1. Introduction

Magnetic resonance imaging [1] (MRI) has recently been used to measure the velocity of chemical waves in several different chemical systems [2–4]. In this paper, it is used to track the reaction front during the gelation of sodium alginate by calcium ions. The sol/gel interface can be visualised in both one and two dimensions as a function of time by MR-images obtained at long echo times (TE) since there is a dramatic reduction in the transverse relaxation time (T_2) of the water protons as the alginate forms a gel [5,6]. This provides a more accurate means of tracking the reaction front than the murexid indicator employed in previous studies by Smidsrød and co-workers [7]. They found that the gelation of

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sodium alginate by calcium ions is limited by the rate of diffusion of the reactants. Using the theory developed by Sherwood and Pigford [8], they were able to suggest a model for the gelation but they did not attempt to derive the diffusion coefficients of the reactants from their experiment results.

In this paper, we have used MRI to track the reaction front during gelation and, applying the same gelation model, have extracted a diffusion coefficient for calcium ions diffusing through calcium alginate gel by assuming that the diffusion coefficient of alginate is negligible. Importantly, from the attenuation in the diffusion coefficient of calcium, a qualitative estimate of pore size in the gel can be made.

2. Results and discussion

Calcium ions react extremely rapidly with alginate to form a gel state, occupying all available binding sites on the alginate molecules; consequently, the reaction front as observed by MRI is at the interface between the calcium alginate gel formed ($T_2 \sim 0.1$ s) and the unreacted sodium alginate solution ($T_2 \sim 0.8$ s).

In Fig. 1, the displacement of the sol/gel interface is visualised by a series of two-dimensional MR-images taken in time. The calcium alginate gel formed when calcium ions (~ 0.06 M) from the outer reservoir diffuse through a dialysis membrane into the central reservoir containing sodium alginate (3 w/v%) has a very short T_2, and thus appears as a dark ring in all the images. The first image [Fig. 1(a)] was acquired 41 min after the sodium alginate solution was placed in contact with the calcium ions, and each subsequent image shows the extent of the reaction at 63-min intervals. Those images have not been used to obtain quantitative information partly because of the finite time ($\sim$ 21 min) it took to acquire each image with optimum contrast, which results in a blurring of the sol/gel interface; and also because of the inherent complexity of the analysis of any diffusion process involving a cylindrical geometry.

To simplify the analysis, the gelation of sodium alginate (2 w/v%) by calcium ions (~ 0.1 M) was arranged to occur in one dimension. The alginate solution was placed in a glass vial (i.d., 11 mm) with a dialysis membrane at one end and inverted over a calcium chloride solution. One-dimensional projections (TE = 100 ms) were taken vertically in the direction of the gel formation to track the displacement of the sol/gel interface at 40-s intervals; little spatial averaging occurred during the data acquisition time as each projection took less than 1 s to acquire. Overall, the gelation process was monitored for a period of $\sim$ 6 h, during which time 512 spatial projections were acquired. The resultant 2D-image shown in Fig. 2 consists of spatial information along the x-axis and time along the y-axis. The narrow reaction zone is expanded to show a parabolic displacement of the front as a function of time; this is characteristic of a diffusion-limited reaction.

Fig. 3 shows four discrete projections taken from the data set of Fig. 2, for different times ($t = 440$, 2440, 4440, and 6440 s). The reaction front is defined to be the point in the projection at which the largest change in intensity occurred [9],

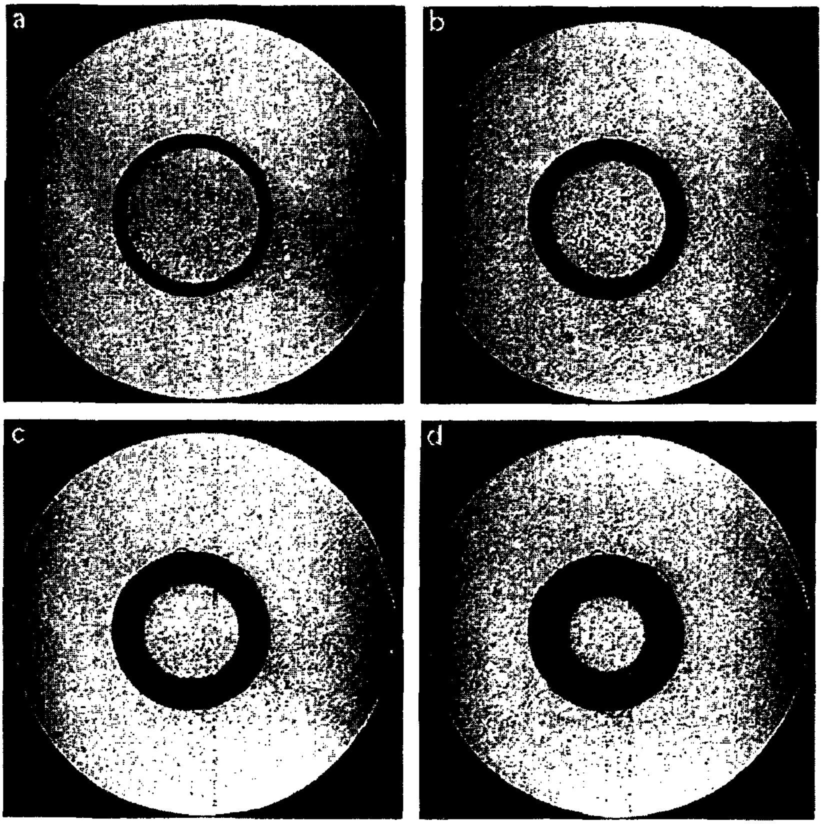

Fig. 1. Two-dimensional gelation of 3 w/v% sodium alginate (central reservoir) by ~ 0.06 M calcium ions (outer reservoir). The calcium alginate gel appears as a dark ring which gradually moves into the sodium alginate reservoir. Images show the extent of gelation after (a) 41 min; (b) 104 min; (c) 167 min; and (d) 230 min. TE, 100 ms; TR, 5 s; field of view (FOV), 5 cm; slice thickness, 5 mm; in-plane resolution, 200 μm; acquisition time, ~ 21 min.

that is between the minimum intensity signal observed for calcium alginate gel and the intermediate intensity signal observed for the sodium alginate solution. The displacement of the reaction front measured from the edge of the image, at 2000-s intervals, gave the plot of displacement vs. (time$^{1/2}$) shown in Fig. 4. This corresponds to the equation:

$$x = 2(\alpha t)^{1/2} + x_0 \qquad (1)$$

where x_0 is the distance of the reaction front at time zero from the edge of the image. The value of α estimated from the slope of the fitted straight line was used in the gelation model to yield the diffusion coefficient of calcium for a given set of reaction conditions. The slope of the fitted straight line, the value of α, the initial reaction conditions, and the derived diffusion coefficient for calcium are given in Table 1, together with the data for experiments repeated for 2, 3, and 4 w/v%

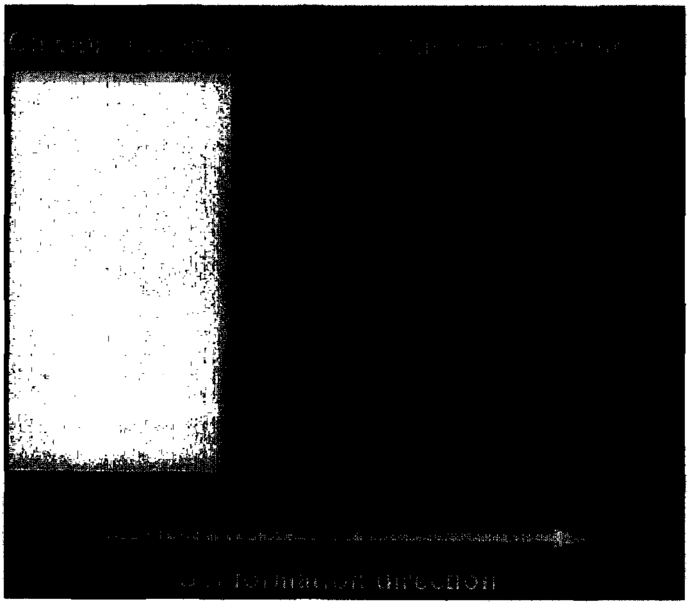

Fig. 2. One-dimensional gelation of 2 w/v% sodium alginate (intermediate intensity) by 0.0976 M calcium ions (high intensity). The calcium alginate gel gives the lowest intensity in the image shown, which consists of a series of 512 projections taken at 40-s intervals. Each projection is represented by 512 pixels of which only 70 are shown. TE, 100 ms; spatial resolution, 160 μm; acquisition time, ~6 h.

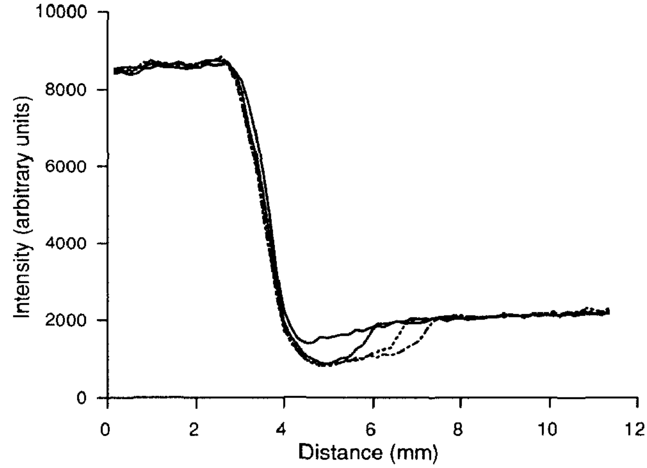

Fig. 3. Four projections taken from the same data set shown in Fig. 2 for different times: 440 s (———), 2440 s (— — —), 4440 s (- - - - -), and 6440 s (– · – · –). The calcium reservoir has the highest intensity, the alginate reservoir has an intermediate intensity, and the calcium alginate gel which is formed has the lowest intensity.

Table 1
Summary of the results for different initial reaction conditions

Alginate (w/v/%)	C_{bi} (mol/L)	C_{ai} (mol/L)	Slope (mm/s$^{1/2}$)×10^2	α (mm^2/s)×10^4	D_a (mm^2/s)×10^4
2.0	0.0698	0.0492	1.88 (0.14) [a]	0.88 (0.13)	1.8 (0.26)
2.0	0.0698	0.0984	3.75 (0.14)	3.52 (0.26)	4.4 (0.32)
2.0 [b]	0.0698	0.0976	3.95 (0.18)	3.90 (0.36)	4.9 (0.36)
2.0 [c]	0.0698	0.0984	4.68 (0.23)	5.48 (0.54)	6.8 (0.54)
3.0	0.1046	0.0492	2.04 (0.25)	1.04 (0.26)	2.8 (0.71)
3.0	0.1046	0.0984	3.21 (0.21)	2.57 (0.34)	4.2 (0.34)
4.0	0.1395	0.0976	3.09 (0.21)	2.39 (0.32)	4.8 (0.32)

[a] The value shown in brackets is the uncertainity at a 95% confidence level. [b] This experiment was used for Fig. 2. [c] The alginate solution contained excess of sodium chloride.

sodium alginate solutions with ~ 0.1 M calcium; 2 and 3 w/v% alginate solutions with ~ 0.05 M calcium; and 2 w/v% alginate solution containing excess of sodium chloride with ~ 0.1 M calcium.

The gelation model first suggested by Smidsrød and co-workers can be used to calculate the diffusion coefficient of calcium ions through the gel network, provided the diffusion coefficient of alginate molecules is assumed to be very low and assuming there is no convective mixing taking place within the calcium alginate gel that is formed [10]. This model, based on the theory developed by Sherwood and Pigford [8], further assumes that the gelation of sodium alginate by calcium ions is stoichiometric, irreversible, and very rapid compared to the rate of diffusion of the reactants; it gives the following equation:

$$\left(\frac{C_{bi}}{2\,C_{ai}}\right)\sqrt{\frac{D_b}{D_a}}\exp(\alpha/D_a)\operatorname{erf}\left(\frac{\alpha}{D_a}\right)^{1/2}=\exp(\alpha/D_b)\operatorname{erfc}\left(\frac{\alpha}{D_b}\right)^{1/2} \qquad (2)$$

where: C_{ai} = initial concentration of calcium ions (mol/L), C_{bi} = initial concentration of sodium alginate (mol/L), α is derived from the slope of the graph of displacement vs. (time$^{1/2}$), D_a = diffusion coefficient of calcium ions through a porous gel (mm^2/s), and D_b = diffusion coefficient of alginate in aqueous solution (mm^2/s). This equation relates the initial reaction conditions C_{ai} and C_{bi} to the physical properties of the system α, D_a, and D_b; a factor of 2 is required for the mass balance at the sol/gel interface because an infinite sheet model is assumed for calcium alginate [11]. The diffusion coefficient of calcium (D_a) can be evaluated, provided the variables C_{ai}, C_{bi}, α, and D_b are known.

The initial concentration of calcium (C_{ai}) is accurately known since the solution is standardised by titration against a volumetric solution of EDTA before use; the value of α can be derived from the slope of the graph of displacement plotted against ($t^{1/2}$) (Fig. 4); the concentration of alginate (C_{bi}) is assumed to be equivalent to the moles of guluronic acid residues per liter, since that residue is responsible for gel formation [12]. To determine the guluronic acid content of commercially available sodium alginate (Fisons), a known mass of sodium alginate solution was added to a volumetric solution of calcium chloride and the residual

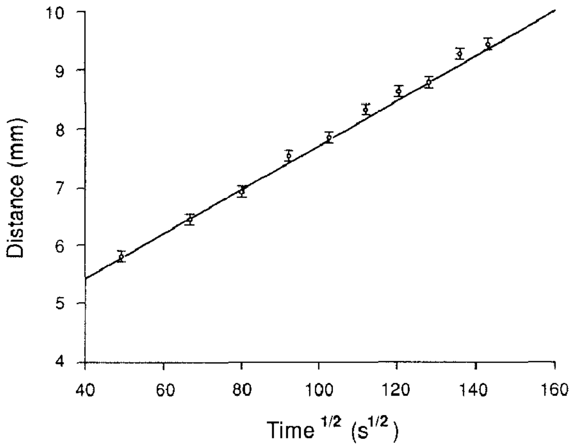

Fig. 4. Plot of displacement of the reaction front against (time$^{1/2}$) for the same data set shown in Fig. 2. The error in the displacement measured from the edge of the image was ±0.08 mm, the slope of the graph was 3.95×10^{-2} mms$^{-1/2}$, and the intercept was 3.83 mm.

calcium chloride concentration was measured by titration. Assuming an infinite sheet model [11] for calcium alginate, where two guluronic acid residues are required to bind each calcium ion, it was found that 1 g of sodium alginate contains ~ 0.0035 mol of guluronic acid which was then used to convert the sodium alginate concentrations (w/v%) into moles per liter of guluronic acid. The only variable that is not known precisely is the diffusion coefficient of the alginate molecules (D_b); it was assumed here that the value of D_b is small compared to the value of α. Consequently, the right-hand side of Eq. *2* may be replaced by an asymptotic expansion given by:

$$\exp(z^2)\operatorname{erfc} z = \frac{1}{\sqrt{\pi}}\left(\frac{1}{z} - \frac{1}{2z^3} + \cdots\right) \tag{3}$$

in which $z = \sqrt{(\alpha/D_b)}$. Assuming that D_b approaches zero, then the right-hand side of Eq. *2* may be replaced by the first term of the expansion given by Eq. *3*; Eq. *4* given below may then be solved for D_a.

$$\left(\frac{C_{bi}}{2\,C_{ai}}\right)\sqrt{\frac{\pi\alpha}{D_a}}\exp(\alpha/D_a)\operatorname{erf}\left(\frac{\alpha}{D_a}\right)^{1/2} - 1 = 0 \tag{4}$$

When the values of C_{ai}, C_{bi}, and α are substituted into Eq. *4*, the value of D_a was determined by Newton's method. The derived diffusion coefficients of calcium ions (D_a) diffusing through a calcium alginate gel in the reaction zone under different reaction conditions are reported in Table 1.

Given the obstructing effect of the gel matrix, as well as the existence of fixed charges on the polymer molecules, it is not surprising that the diffusion coefficient of calcium was found to be less than that of calcium ions in free solution [13]. The results in Table 1 show that different experimental conditions resulted in a range of values for the derived diffusion coefficient of calcium ions. The diffusion coefficient of calcium ions at low concentrations of calcium ion is much lower than that for high concentrations of calcium ion, whereas the concentration of the alginate reservoir did not appear to have much effect on the value of D_a. Furthermore, excess of sodium chloride resulted in an increase in the diffusion coefficient of calcium. Those results can be explained by considering the two main mechanisms which influence the diffusion of calcium ions through calcium alginate gel; namely, the electrostatic interactions that occur between the fixed charges on the polymer matrix and the diffusing ions, and the obstructing effect of the gel matrix [10]. The concentration dependence of D_a confirms that there is some non-specific binding of calcium ions to the polymer chains. Assuming that a fraction (x) of the diffusing ions are bound to the polymer, then the apparent diffusion coefficient of the calcium ions will be

$$D = (1 - x) D_{\text{free}} + x D_{\text{bound}} \tag{5}$$

where $D_{\text{free}} \gg D_{\text{bound}}$. Therefore, the overall diffusion coefficient of calcium ions is governed by the fraction of freely diffusing calcium ions; as the concentration of calcium increases, the binding sites become saturated and more calcium ions are free to diffuse, and hence the diffusion coefficient of the calcium ions increases. The addition of excess of sodium chloride swamps the fixed charges on the polymer molecule and so less calcium ions are involved in non-specific binding, which results in a much higher diffusion coefficient for the calcium ions.

The reduction in the measured diffusion coefficient of the calcium ions can also be related to the volume fraction of polymer (v_p) in the gel, using Mackie and Meare's equation [14]:

$$\frac{D_a}{D_{ao}} = \left[\frac{1 - v_p}{1 + v_p}\right]^2 \tag{6}$$

which ignores the electrostatic interactions between the gel network and the diffusing ions. The value of D_{ao} is the diffusion coefficient of calcium ions in the absence of the gel matrix. To calculate the volume fraction of polymer in each gel made under different reaction conditions, the value of D_{ao} was assumed to be 7.9×10^{-4} mm^2/s, which is the self-diffusion coefficient of calcium ions at infinite dilution [15]. This assumption is adequate since the calcium ions in the reaction zone diffuse under a very small chemical potential which falls to zero at the sol/gel interface; this is similar to the conditions under which the self-diffusion coefficient of calcium ions is measured.

The polymer volume fractions calculated by Eq. *6* are presented in Table 2. It appears that gels containing no salt have a higher volume fraction of polymer compared to the gel made with excess of salt. This may be explained by the fact that the packing of alginate molecules depends on the molecular conformation of the alginate molecules. In the presence of salt, alginate molecules exist as random coils [16,17] which, on forming a gel, cannot pack together very tightly. In the absence of salt, the alginate molecules have rod-like structures [17] which can pack

Table 2
Volume fraction of alginate in gels formed under different reaction conditions [a]

C_{ai} (mol/L)	C_{bi} (mol/L)	D_a (mm^2/s)×10^4	υ_p
0.0492	0.0698	1.8	0.36
0.0984	0.0698	4.4	0.15
0.0976	0.0698	4.9	0.12
0.0984 [b]	0.0698	6.8	0.04
0.0492	0.1046	2.8	0.25
0.0984	0.1046	4.2	0.16
0.0976	0.1395	4.8	0.12

[a] The value of D_{ao} was 7.9×10^{-4} mm^2/s. [b] The alginate solution contained excess of sodium chloride.

together tightly; however, the fixed charges on the alginate molecules allow for a defined packing density and hence changes in the concentration of alginate should not affect the volume fraction of polymer in the gel significantly. The polymer volume fraction in gels made from low concentrations of calcium ion was very high, which suggests that electrostatic interactions between the fixed charges on the polymer and the diffusing calcium ions cannot be ignored. Thus, it can be concluded that diffusion of calcium ions in gels made from low concentrations of calcium is governed by electrostatic interactions, whereas diffusion in gels made from high concentrations of calcium is influenced mainly by the gel structure.

3. Conclusions

The above results demonstrate that MRI can be used to track the reaction front during the gelation of sodium alginate by calcium ions. From the theory of moving boundaries, a diffusion coefficient of calcium ions through the reaction zone (D_a) can be calculated, and the value of D_a obtained was used to gain some insight into the gel structure that is formed.

It was shown that the diffusion of calcium ions through a calcium alginate gel depends on the concentration of calcium ions in the reservoir, and hence on the extent of the electrostatic interactions as well as the pore size of the gel. The addition of salt swamps the non-specific binding sites, resulting in a near-neutral gel matrix through which calcium diffuses; hence, the reduction in the diffusion coefficient of calcium is mainly due to obstruction from the gel network.

Given existing chemical knowledge, coupled with the new insights available from these studies, it should now be possible to tailor-make a gel with specific controlled-release characteristics from a given set of reaction conditions and, from the reduction in the diffusion coefficient of calcium ions, to obtain a qualitative estimate of pore size from the polymer volume fraction.

Finally, it should be stated that this technique together with the null-point technique [18] can be used to study the diffusion of any substrate that has associated with its presence, or its reaction with a local chemical substrate, a means to cause a change in the relaxation time of water or any other NMR-active species.

4. Experimental

Samples.—A ~ 0.1 M stock solution of $CaCl_2 \cdot 6H_2O$ (BDH) was standardised against 0.1 M EDTA (BDH), using Solochrome Black (BDH) as an indicator [19]; all subsequent calcium chloride solutions were prepared by diluting this stock solution. Aqueous solutions of sodium alginate (Fisons) were prepared by dissolving a known mass in a known volume of distilled water; the solutions were homogenised to ensure good mixing and then centrifuged to remove incorporated air bubbles.

Gelation experiments.—The one-dimensional reaction vessel consisted of a test tube (i.d., 11 mm) containing an aqueous solution of sodium alginate, with a dialysis membrane over the open end, inverted in a solution of calcium chloride contained in a larger vial. Time zero was when the two solutions were placed in contact with each other. The reaction vessel used in the two-dimensional gelation experiment consisted of a length of dialysis tubing (diameter, 20 mm; length, ~ 4 cm) filled with a 3 w/v% sodium alginate solution and sealed at both ends; this was placed vertically in a beaker of calcium chloride solution (~ 0.06 M) at time zero.

MRI instrumentation.—All MRI experiments were conducted using an Oxford Research Systems BIOSPEC I imaging spectrometer coupled to an Oxford Instruments 310-mm horizontal bore, superconducting magnet operating at 2.0 T (83.7 MHz for ^1H). Magnetic field gradients for imaging in three orthogonal directions were generated by a custom-built Helmholtz-Golay gradient set [20] (i.d., 200 mm) and all samples were studied using a split-ring resonator probe [21] (i.d., 60 mm; height, 115 mm) placed with its long axis vertical in the bore of the gradient set.

One-dimensional experiments.—Projection images of the 1D gelation process were obtained using a Hahn spin-echo sequence [22], with an echo time of 100 ms, and a pulsed magnetic field gradient (1.0 Gauss/cm) oriented perpendicular to the reaction front; an echo time of 100 ms provides the best contrast between the alginate sol and the gel that is formed. The gelation process was monitored for ~ 6 h during which time 512 projection images were acquired. The resultant two-dimensional image encodes spatial information along the gel formation direction (x-axis), with time as the other dimension (y-axis); although each projection image represents a 8.0-cm field of view with a spatial resolution of 160 μm along the cylindrical axis of the reaction vessel, only 1.1 cm of the reaction zone is shown in Fig. 2.

Two-dimensional experiment.—The two-dimensional gelation process was imaged by two-dimensional slice imaging, using a conventional "spin-warp" imaging sequence [23] that utilises a 3-lobed slice-selective 180° sinc pulse; the recycle delay (TR) was 5 s and the TE was set to 100 ms. The gelation process was monitored for ~ 6 h during which time 16 images were acquired of which only four are shown

(Fig. 1). Each slice image, represented by 256 × 256 pixel elements, had a slice thickness of 5 mm, and a field of view of 5 cm corresponding to an in-plane resolution of ~ 200 μm. The total acquisition time for each image was ~ 21 min.

Computing.—All image data were processed on a Sun 4/150 TAAC workstation, using in-house "CaMReS" software (by Dr. Nick Herrod). Fitted straight lines were obtained by least-squares linear regression. Calcium diffusion coefficients were calculated using Mathematica, a commercially available software package, operating on a NeXT computer.

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